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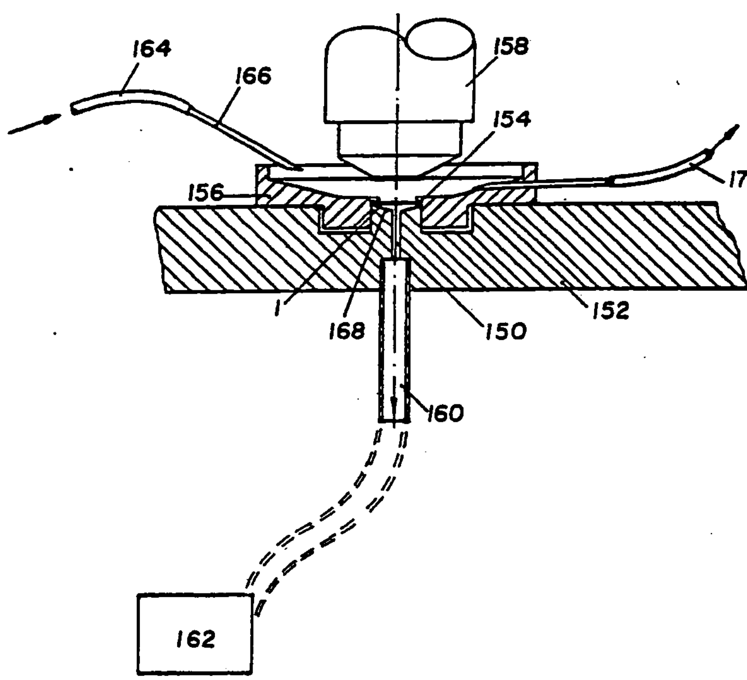

(57) Abstract

Individual living cells are placed at identifiable locations through the use of a carrier having a plurality of apertures which are 1) arranged in an ordered array, and 2) sized to hold individual cells. Once in the apertures, the cells can be studied, examined or manipulated on a one-by-one basis.

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SYSTEM AND METHODS FOR CELL SELECTION

The present invention relates to equipment and methods for cell selection and, more particularly, to equipment and methods for trapping individual cells at known locations. More generally, the invention relates to equipment and methods for studying, examining, or manipulating large groups of living cells, e.g., 10,000 or more individual cells, on a cell-by-cell basis.

In Swiss patent application Serial No. 2897/82-3, filed May 10, 1982, the relevant portions of which are incorporated herein by reference, we described equipment and methods for studying living cells on a cell-by-cell basis.

Briefly, our Swiss application described a process for placing individual living cells at identifiable locations comprising the steps of:

(a) providing a carrier having a plurality of apertures, the apertures being arranged in an ordered array and being sized to hold individual cells;

(b) applying a fluid containing living cells to the carrier; and

(c) applying a force to the cells to move the cells into the apertures.

Once in their individual apertures, the cells are studied, examined, and manipulated on a one-by-one basis. For example, the cells in the carrier apertures can be subjected to biological tests and particular properties of individual cells can be measured. As described in detail in our Swiss application, a particularly important application of this analysis approach involves using it to perform the Cercek SCM (Structuredness of Cytoplastic Matrix) test for diagnosing cancer. See, for example, L. Cercek et al, Biophys. J., July 1978, Vol. 23, No. 1, p. 395 ff.

The present invention relates to new equipment and methods for practicing and using the methods and equipment described in our prior Swiss patent application. In particular, the objects of the present invention include providing improved methods and equipment for

loading cells onto a carrier and preventing them from leaving a carrier; improved methods and equipment for exchanging the bathing fluid surrounding cells captured in the apertures of a carrier; improved methods and equipment for selecting and removing particular cells from a carrier; improved methods and equipment for washing excess cells from a carrier; and improved carriers and methods for constructing such carriers.

To achieve the foregoing and other objects, the invention in accordance with certain of its aspects provides equipment and methods for placing individual living cells at identifiable locations on a cell carrier wherein electromagnetic fields are used for loading the cells onto the carrier. In certain embodiments of these aspects of the invention, crossed electric and magnetic fields are used for loading, while in other embodiments, an electric field normal to the surface of the carrier is used.

In accordance with other aspects of the invention, a time varying magnetic field is used to enhance the rate at which bathing fluid surrounding cells captured in the apertures of a carrier is exchanged. In addition to enhancing the rate of fluid exchange, such a time varying magnetic field also has a massaging effect on the cells captured in the apertures.

In accordance with additional aspects of the invention, cells are held in the apertures of a carrier by adjusting the osmolarity of the bathing solution surrounding the cells so as to cause the cells to swell.

In accordance with further aspects of the invention, electromagnetic fields are used to select and remove particular cells from a cell carrier. In certain embodiments of these aspects of the invention, a time varying electric field and a constant magnetic field is used to select and remove cells having a particular charge to mass ratio. In other embodiments, a charged probe is used to remove individual cells from the carrier.

In accordance with additional aspects of the invention, equipment and methods are provided for washing excess cells from the surface of a cell carrier wherein a pressure differential is applied across the cell carrier during the washing process. In certain preferred embodiments of these aspects of the invention, the carrier is

washed by supplying fluid to its top surface through an inflow tube and removing it through a drain tube.

In accordance with still further aspects of the invention, improved cell carriers and methods for producing such carriers are provided. In particular, the invention provides coated cell carriers and cell carriers having apertures which include at least one vertical wall. The latter carriers are conveniently prepared using an ion bombardment process.

Further objects and features of the present invention will become more fully apparent from the following description of several embodiments of the invention based on the accompanying drawings, wherein:

Figs. 1A-1E are schematic illustrations, partly in sectional view, of preferred cell carriers of the invention.

Figs. 2-4 are scanning electron micrographs of copper carriers for use with the present invention.

Fig. 5 is a scanning electron micrograph showing a copper carrier coated with silicon.

Fig. 6 is a scanning electron micrograph showing a copper carrier having square-shaped apertures which are sized to hold and retain lymphocytes having a cross-sectional size of approximately 7 um.

Fig. 7 shows a typical experimental arrangement suitable for loading cells into carriers of the types shown in Figs. 2-4.

Fig. 8 is a scanning electron micrograph showing a carrier filled with lymphocytes.

Figs. 9-10 are scanning electron micrographs showing individual cells in individual apertures of a carrier.

Fig. 11 is a scanning electron micrograph showing the surface of the carrier prior to washing.

Fig. 12 shows the use of an electric field to drive cells into the apertures of a carrier.

Fig. 13 shows the use of crossed electric and magnet fields to drive cells into the apertures of a carrier.

Figs. 14-15 show the use of a time varying magnetic field to enhance fluid exchange about cells captured in a carrier.

Fig. 16 shows the use of a time varying E field crossed with a constant B field to select a sub-population of cells captured in a carrier based on their charge to mass ratio.

As indicated above, the present invention relates to improved equipment and methods for studying, examining or manipulating living cells on a one-by-one basis wherein individual living cells are placed at identifiable locations on a cell carrier.

The cell carrier has an array of cell receiving holes, where for each hole, the location in the array, or address, is fixed and known. The holes extend from the carrier top side to a spaced apart bottom side. The holes have preselected configurations so that when a batch of cells passes over the carrier top side only preselected cells, based on their particular size, enter and become supported in the holes. Cells of sizes smaller than those of the selected cells pass through the holes, while much larger cells cannot enter the holes. Once the carrier is rinsed, only selected cells are located in its holes, one cell per hole at a fixed address.

Various cell carrier configurations are shown in Figs. 1A-1E. Carrier 1 includes base 3 in which are formed apertures or holes 2. The apertures or holes, as well as their arrangement, may have various configurations. In Fig. 1A, the holes are arranged in rows and columns along axes X and Y, respectively. As shown in Fig. 1B, the holes have larger openings at their tops than at their bottoms. The side walls of the apertures may converge continuously towards the opening at the bottom side 1b of the cell carrier, or in steps, as shown in Fig. 1C. Also, as shown in Fig. 1D, not all sides of the aperture need slope inwardly. Rather, a portion of the walls of the aperture can be essentially vertical so as to help capture and retain the cells in the apertures, especially when the cells are introduced into the carrier by being flowed across the top of the carrier in a direction substantially perpendicular to the apertures' vertical walls.

The shape of apertures 2 enables the cells to be effectively held to the carrier by applying means, such as a pressure difference between the upper and the bottom side of the carrier, or electromagnetic forces. Briefly, to first separate a particular group of cells from cells of other groups, since the cells in each

group are of known size or sizes, which typically differ from those in other groups, the carrier 1 is chosen to have holes of sizes so that when the matter, e.g., blood, containing the various cell groups is placed on the carrier 1, effectively most if not all of the holes are occupied by cells of the group of interest, one cell per hole.

For example, with regard to the SCM test referred to above, the holes are sized to be suited for receiving lymphocytes, among which there are two main sizes of about 7 um and about 10-15 um, the 7 um lymphocytes being the cells of interest. To capture and retain this population of cells, it has been found that at the upper surface or side 1t of carrier 1, the apertures should have a cross-sectional dimension of approximately 10 um and that at the bottom surface or side 1b, they should have a cross-sectional dimension of approximately 5 um. In this way, the desired population of cells can easily enter the aperture without suffering substantial damage and yet, once in the aperture, the cells cannot pass out of the bottom of the carrier.

In general the aperture should be shaped so that either at its bottom side or at a cross-section intermediate sides 1t and 1b, the cross-sectional dimension is less than at the top side, so that a desired cell entering an aperture does not pass through the aperture, but rather is held therein. Fig. 1E illustrates an aperture configuration wherein the minimum cross-section is located in a plane intermediate between the top and bottom sides of the carrier. In addition to properly selecting the aperture's entering and exiting dimensions, it is also important to choose the carrier thickness between the top and the level of the minimum cross-sectional dimension so that the size of the aperture is related to the size of the desired cells so that when a desired cell enters an aperture practically the entire cell is within the aperture, thus preventing it from being washed out during washing of the carrier.

The carrier 1 is made of any appropriate matter, e.g., metals such as copper, gold, nickel, silver or others, or of plastic.

In addition to using pure metal or plastic carriers, in some cases it is desirable to coat the carrier with various materials in order to change either or both of its chemical and mechanical surface characteristics. Examples of suitable coating materials include

silicon, silicon dioxide and various inorganic glasses. When using such coating materials, or for that matter, when choosing a material from which to make an uncoated carrier, it is important to determine that the material does not interact with the cells in a way which will interfere with the test or tests to be performed.

For example, with regard to the SCM test referred to above, it has been found that a coating of SiO_2 on the carrier leads to activation of the cells (lymphocytes) which masks the response of these cells to stimulating agents. A similar activation is found with a mixture of silicon and Si_2O_3. Pure silicon, on the other hand, does not lead to activation of the cells. Accordingly, for the SCM test, a carrier coating of silicon is appropriate, while a coating of silicon dioxide or silicon plus Si_2O_3 is not. Similar selections of coating materials can be readily made by persons skilled in the art for other types of diagnostic tests.

Scanning electron micrographs of a copper carrier for use with the present invention are shown in Figs. 2-4. Fig. 2 shows the top surface of the carrier at a magnification of 1000 X. At the level of this surface, the apertures have a cross-sectional dimension (diameter) of approximately 11 microns. The minimum cross-sectional dimension for these apertures is located in a plane intermediate the carrier's top and bottom surfaces and has a magnitude of approximately 4 microns. The spacing between this intermediate plane and the top surface of the carrier is approximately 6 microns. The spacing between apertures is approximately 15 microns. In general, the inter-aperture spacing should be kept as small as possible so as to maximize the chances that cells will come to rest inside apertures rather than on the portions of the carrier between apertures.

Figs. 3 and 4 show the bottom surface of the carrier of Fig. 2 at a magnification of 1000 X. Fig. 3 also shows a turned-up corner of the carrier. Examining the edges of the carriers of Fig. 2-4 reveals that the apertures have a vertical cross-sectional configuration of the type shown in Fig. 1E.

The carrier shown in Figs. 2-4 was prepared using a standard photo-etching technique of the type commercially employed to make transmission electron microscope grids. As is known in the art, that process, in its last stages, involves the deposition of metal on one

side of a preformed grid so as to increase the strength of the grid. When a transmission electron microscope grid is to be formed, the deposition step is carried on only for a short time so as to keep the size of the apertures as large as possible, i.e., to minimize the width of grid members which in turn minimizes the interference of the grid with the transmission of electrons through the specimen and to the electron detector. To form the carrier of Figs. 2-4, the deposition step, rather than being short, was continued for a relatively long period of time until enough metal was deposited on the back surface of the grid to fill in the apertures to the extent shown in the figures. As shown most clearly in Fig. 4, the deposited metal (copper) built up on the solid parts of the grid and overlapped into the apertures to close off the apertures and thus form the desired minimum cross-sectional dimension of the apertures.

Rather than using a deposition process to form carrier apertures of the desired configuration, other processes, in particular ion bombardment processes through masks of different thicknesses and the like, can be used. Such processes are particularly useful in preparing asymmetric apertures, such as the apertures shown in Fig. 1D.

Fig. 5 is a scanning electron micrograph at a magnification of 720 X illustrating a coated carrier. The base carrier in this case was formed from copper and the coating is pure silicon which was deposited on the carrier by vapor deposition. As can be seen in Fig. 5, coatings can be used to change (reduce) the cross-sectional dimensions of the apertures, as well as to provide an especially smooth and/or inert surface for contacting the cells.

Fig. 6 shows another uncoated, copper carrier, in this case having square rather than circular apertures. The cross-sectional dimension of the apertures at the top surface of this carrier is approximately 10 microns and the minimum cross-sectional dimension is approximately 5 microns. The minimum cross-sectional dimension lies in a plane approximately 7-8 microns below the top surface of the carrier. The spacing between apertures is approximately 12 microns.

The carriers of Figs. 2-4 and 6 are sized to be particularly well suited to capturing and retaining lymphocytes having a cross-sectional size of approximately 7 um. As will be evident to persons skilled in the art from the disclosure herein, other carriers

having different aperture configurations can be constructed for capturing and retaining cells of different types and sizes.

As previously pointed out, the holes 2 in carrier 1 are regularly arranged over or in the carrier, e.g., in rows and columns, to enable a clear identification of the position of very hole 2, for example, by its X and Y coordinates in the plane of the carrier. In the described embodiment the holes are disposed in rows and columns, extending perpendicularly to each other, thereby forming a matrix-like structure. The number of holes is chosen depending on the number of cells to be carried. For example, with 100 holes per row and column there is a total of 10,000 holes to carry 10,000 cells on the carrier of the described embodiment, each with its unique position in X and Y.

To practice the method of the present invention, a few drops of the solution containing the cells, e.g., blood containing the lymphocytes, are dripped onto the cell carrier. A force, for example, a pressure differential, is applied across the carrier to move the cells into the apertures. The liquid passes through the holes in the carrier. However, the cells remain on the carrier. Since the sizes of the holes 2 are chosen to accommodate lymphocytes only, they enter the holes. Each hole accommodates only one cell. Excessive and other cells may be washed off the surface of the carrier, such as cells of sizes so great that they can't enter any hole, and/or excess cells more than the number of holes. Thereafter, in order to prevent the cells in the holes from leaving the carrier, they may be fixed thereto by various means, e.g., by applying a continuous pressure differential across the holes, by changing the osmolarity of the bathing solution to cause the cells to swell, by covering the carrier by an adhesive, colloidable matter, and by electrically charging the carrier, as well as by external electric and/or magnetic fields.

Fig. 7 illustrates a typical experimental arrangement which has been used to load cells into carriers of the types shown in Figs. 2-6.

Carrier 1 is held in place above orifice 150 in plate 152 by means of collar 154 of solution basin 156. The collar presses the carrier against the portion of plate 152 which surrounds orifice 150 and creates a seal between that portion and the carrier. This seal

prevents substantial numbers of cells from passing around the edges of the carrier, rather than being captured in the apertures.

Orifice 150 is connected by outflow tube 160 to pump 162. The pump serves to produce a pressure differential across carrier 1 which pulls the cells into the apertures in the carrier. It has been found that a more uniform filling of carrier 1 can be achieved by providing a shallow taper 168 at the mouth of orifice 150. This taper reduces the amount of time required to fill the apertures at the perimeter of the carrier.

Basin 156 is configured so as to allow microscope objective 158 to be brought close enough to carrier 1 so that the apertures in the carrier can be brought into focus. Solutions are provided to basin 156 by one or more inflow tubes 164 which are conveniently connected to syringe needles 166. The inflow tubes are used to introduce various bathing and reagent solutions to basin 156. The inflow tubes are also used to wash excess cells off the top surface of carrier 1. In this case, fluid is removed from basin 156 by means of drain tube 170. Cells are applied to carrier 1 using a standard syringe. During this operation microscope objective 158 and basin 156 are moved apart to allow ready access to carrier 1. The level of fluid in basin 156 is monitored during the testing of cells and, as necessary, fluid is added to the basin to keep the cells captured in carrier 1 continuously submerged in liquid.

A typical procedure used to capture and retain human lymphocytes in a carrier using the apparatus of Fig. 7 was as follows. First, a sample of human whole body was obtained in the standard way. A plasma fraction of this blood was then obtained by either centrifuging the sample at approximately 100 g for approximately 6 minutes or by incubating the sample at 37°C for approximately a half an hour. In either case, the plasma fraction had a pinkish cast indicating the presence of red blood cells. The red blood cell/white blood cell ratio of the plasma fractions used to fill carriers was estimated to be approximately 30/1.

Once obtained, the plasma fractions were diluted with phosphate buffered saline until a cell concentration of either approximately 6 x 10^6 cells/cc or 12 x 10^6 cells/cc was reached. So that the cells would fluoresce and thus be easily seen under the microscope during

loading onto the carrier, fluorescein diacetate (FDA) was added to the cell suspension at a concentration of approximately 2.5 uM and the cells were incubated with FDA for approximately 10-30 seconds prior to being applied to the carrier.

To make sure that the carrier was free from contamination, phosphate buffered saline was added to basin 154 with carrier 1 in place and pumped through carrier 1 by means of pump 162. Pump 162 was adjusted to produce a pressure differential across carrier 1 in the range of 0.5-5.0 cm of water.

The cells were applied to carrier 1 by bringing a standard syringe containing the cell suspension into the vicinity of the carrier. For the 6 x 10^6 cells/cc concentration it was found that three drops of the cell suspension applied near to, but not directly on, the carrier were adequate to essentially fill all of the apertures in a carrier having approximately 7500 holes, while for the 12 x 10^6 cells/cc concentration level and the same size carrier, one drop applied directly to the carrier was found to be sufficient. In either case, essentially complete filling of the carrier occurred within a period of seconds to minutes, depending on how well collar 154 sealed the carrier to plate 152.

Fig. 8 is a scanning electron micrograph at a magnifications of 1000 X showing the carrier filled with lymphocytes. The fixation process used to prepare this micrograph causes the cells to contract. This makes them appear somewhat smaller than the apertures. When the cells were alive, they essentially filled the whole aperture with their tops at or just below the top surface of the carrier.

Figs. 9-10 are scanning electron micrographs at a magnification of 6600X showing individual cells in individual apertures. The cell shown in Figs. 9 is a lymphocyte, while the cell in the aperture in Fig. 10 is an erythrocyte. Because erythrocytes are smaller than lymphocytes and are relatively flexible, if pressure had continued to be applied across the carrier, the erythrocyte shown in Fig. 10 would have passed down and out of the aperture.

Fig. 11 is a scanning electron micrograph at a magnification of 480X showing the carrier surface prior to washing to remove excess cells and debris. For the apparatus of Fig. 7, washing is done using inflow tube 164 and drain tube 170. Note that the pressure

differential created by pump 162, as well as the configuration of the apertures, serves to hold the cells in their apertures during the washing process.

As can be seen in Fig. 11, prior to washing there are individual lymphocytes in individual apertures, but the top of the carrier is covered with both excess lymphocytes and erythrocytes, as well as other cell types and debris. Comparing Fig. 11 with Fig. 8, which shows the carrier surface after washing, clearly demonstrates the effectiveness of the washing process in removing excess cells and debris.

In the examples described above, a pressure difference across carrier 1 has been used to drive the cells into the carrier apertures and then retain the cells in the apertures. Other forces can also be used for these purposes.

For example, Fig. 12 shows the use of an electric field to drive the cells against the carrier and into the apertures. The field is oriented perpendicular to the top surface of the carrier. As is known in the art, biological cells, including lymphocytes, normally carry a net electrical charge, or, by adjusting the pH or other parameters, can be made to have a net charge. The electric field shown in Fig. 12 will accordingly cause cells, e.g., positively charged cells, to move towards the carrier and into the apertures, as desired. Of course, if it is negatively charged cells which one wants to capture on the carrier, one only needs to reverse the direction of the electric field.

The use of an electric field as the driving force can lead to electrolysis problems with uncoated metallic carriers. One solution to this problem is to coat the carrier with a non-conductor, as described above. Another solution, illustrated in Fig. 12, is to give the carrier a shape which localizes most of the electrolysis effects at points distant from the apertures where the cells are captured. In particular, in Fig. 12, the carrier is provided with ears or projections 172 which concentrate the electric field and thus the ionic current and electrolysis effects in regions away from the main body of the carrier. Such ears will also attract cells, but in general there will be an abundant excess of cells so that even if there is some concentration of cells in the regions of the ears, there will

still be enough cells near the body of the carrier to fill the apertures.

As an alternative to using an electric field oriented perpendicular to the surface of the carrier, crossed electric and magnetic fields parallel to the surface of the carrier can be used to drive the cells into the apertures. As shown in Fig. 13, in this case, the electric field causes the charged cells to move across the top of the carrier, while the magnetic field produces a v x B force towards the surface of the carrier for positively charged cells. Again, negatively charged cells can be selected by reversing the direction of the B field. The use of a B field to drive the cells into the apertures has the advantage that once the cell comes to rest in the aperture, the force on the cell due to the driving force ceases because v is now equal to zero. In contrast, a pressure differential driving force continues to exert a force on the cells even after they have been captured in apertures, although in general this force is too small to cause damage to the cells.

In addition to using E and B fields to apply cells to the carrier, these fields can be used to enhance the rate of fluid exchange around individual cells and to select specific cells captured on the carrier based on such parameters as the cell's charge to mass ratio.

With regard to fluid exchange, Fig. 14 shows the use of a time varying magnetic field normal to the surface of the carrier to cause cells to rotate about their axes inside apertures. More specifically, the time varying magnet field generates a circular or tangential electric field parallel to the plane of the carrier. The magnitude and direction of such a field is described by Maxwell-Faraday law, also known as Lenz's law. This field acts on the fixed charges on the surface of the cell membrane and thus causes the cells to rotate about an axis parallel to the magnetic field. It should be noted that once the cells begin to rotate their cell membranes will experience either an inward or outward squeezing force resulting from the v x B (Lorentz) interaction between the charges on the membrane and the applied B field (see Fig. 15). Whether the force is inward or outward will depend on the sign of the cell's surface charge and the orientation of the B field. In essence, the time varying magnetic field, in addition to rotating the cells, will also have a massaging

effect on them. Furthermore, there will be a tendency for the rotating cell, which in effect is a magnetic dipole, to move parallel to the magnetic field. In addition to the effects on the cells, the field also interacts with the charged ions in the bathing medium causing them to move in circular paths.

With regard to selecting particular types of cells from among the population captured on the carrier, Fig. 16 shows an arrangement for selecting those cells having a particular charge to mass ratio. As shown in that figure, a time varying, e.g., sinusoidal, electric field is applied across the carrier and a constant magnetic field is applied parallel to the top surface of the carrier. The response of individual cells to the electric field will depend on the frequency of the field and the cell's charge to mass ratio. Accordingly, by varying the frequency of the electric field, specific subgroups of cells can be made to move sufficiently far out of their apertures so that the force due to the magnetic field acting on the moving cell will cause it to move in the plane of the surface of the carrier. By means of surface washing during this process, these selected cells can be removed.

In addition to the foregoing, electric fields can be used to select individual cells. For example, individual cells can be removed from the carrier by a local electric field created by bringing a charged probe into the vicinity of a particular cell's aperture. Groups of cells can be similarly removed from the carrier and moved to a desired location by using a movable array of probes, where selected probes in the array can be charged to a value sufficient to attract and move a cell from its aperture.

Although particular embodiments of the invention have been described and illustrated herein, it is recognized that modifications and variations may readily occur to those skilled in the art, and consequently, it is intended that the claims be interpreted to cover such modifications and equivalents.

What is claimed is:

1. A method for placing individual living cells at identifiable locations comprising the steps of:

(a) providing a carrier having a plurality of apertures, the apertures being arranged in an ordered array and being sized to hold individual cells;

(b) applying a fluid containing living cells to the carrier; and

(c) applying an electromagnetic force to the cells to move the cells into the apertures.

2. The method of Claim 1 wherein the electromagnetic force is produced by an electric field oriented perpendicular to the surface of the carrier.

3. The method of Claim 1 wherein the electromagnetic force is produced by crossed electric and magnetic fields oriented parallel to the surface of the carrier.

4. Apparatus for placing individual living cells at identifiable locations comprising:

(a) a carrier having a plurality of apertures, the apertures being arranged in an ordered array and being sized to hold individual cells; and

(b) means for applying an electromagnetic force to the cells to move the cells into the apertures.

5. The apparatus of Claim 4 wherein the means for applying an electromagnetic force includes means for producing an electric field oriented perpendicular to the surface of the carrier.

6. The apparatus of Claim 5 wherein the carrier is made of metal and is coated with a non-conductor.

7. The apparatus of Claim 5 wherein the carrier is made of metal and has a configuration which localizes electrolysis effects at points distant from the locations of the apertures.

8. The apparatus of Claim 7 wherein the carrier includes projections which extend away from the portion of the carrier which includes the apertures.

9. The apparatus of Claim 4 wherein the means for applying an electromagnetic force includes means for producing crossed

electric and magnetic fields oriented parallel to the top surface of the carrier.

10. In a method for exchanging the bathing fluid surrounding cells captured in the apertures of a carrier, the improvement comprising applying a time varying magnetic field normal to the surface of the carrier to cause the cells to rotate about their axes inside the apertures.

11. Apparatus for exchanging the bathing fluid surrounding cells captured in the apertures of a carrier comprising:

(a) means for applying and removing bathing fluid from the carrier; and

(b) means for producing a time varying magnetic field normal to the surface of the carrier to cause the cells to rotate about their axes inside the apertures.

12. In a method for holding cells in the apertures of a carrier, the improvement comprising washing the carrier to remove excess cells and debris, and then adjusting the osmolarity of the bathing solution surrounding the cells so as to cause the cells to swell.

13. A method for selecting cells which have a particular charge to mass ratio from among a population of cells captured in the apertures of a carrier comprising the steps of applying a time varying electric field across the carrier and applying a constant magnetic field parallel to the surface of the carrier to move cells having the particular charge to mass ratio out of their apertures.

14. The method of Claim 13 including the additional step of washing the surface of the carrier while said electric and magnetic fields are being applied.

15. Apparatus for selecting cells which have a particular charge to mass ratio from among a population of cells captured in the apertures of a carrier comprising first and second means for respectively producing electric and magnetic fields, said electric field being time varying and being directed across the carrier, said magnetic field being constant and being directed parallel to the surface of the carrier, said electric field causing cells which have the particular charge to mass ratio to move sufficiently far out of their

apertures so that said magnetic field can move the cells in the plane of the surface of the carrier.

16. The apparatus of Claim 15 further comprising means for washing the surface of the carrier.

17. A method for removing an individual cell from an aperture formed in a carrier comprising the steps of charging a probe and subjecting the cell to the local electric field produced by the charged probe to move the cell out of its aperture.

18. The method of Claim 17 wherein a group of individual cells are removed from their apertures by charging an array of probes and subjecting the cells to the local electric fields produced by the array to move the cells out of their apertures.

19. Apparatus for removing a cell from an aperture formed in a carrier comprising a probe and means for charging the probe to a value sufficient to attract and move the cell from its aperture.

20. Apparatus for removing a group of cells from the apertures of a carrier comprising an array of probes and means for charging selected probes in the array to a value sufficient to attract and move a cell from its aperture.

21. In a method for washing excess cells and debris from a carrier which has a plurality of apertures which are filed with cells, the improvement comprising applying a pressure differential across the carrier to hold the cells in the apertures during the washing process.

22. The method of Claim 21 wherein the washing is performed by supplying fluid to the top surface of the carrier through an inflow tube and removing it through a drain tube.

23. A carrier for holding individual cells at identifiable locations comprising a base made of a first material and having a plurality of apertures therethrough, said apertures being arranged in an ordered array and being sized to hold individual cells, and a coating made of a second material and covering at least a portion of said base.

24. The carrier of Claim 23 wherein the base is a conductor and the coating is a non-conductor.

25. The carrier of Claim 24 wherein the base is metal and the coating is an inorganic glass.

26. The carrier of Claim 24 wherein the base is metal and the coating is Si, SiO_2, or a mixture of Si and Si_2O_3.

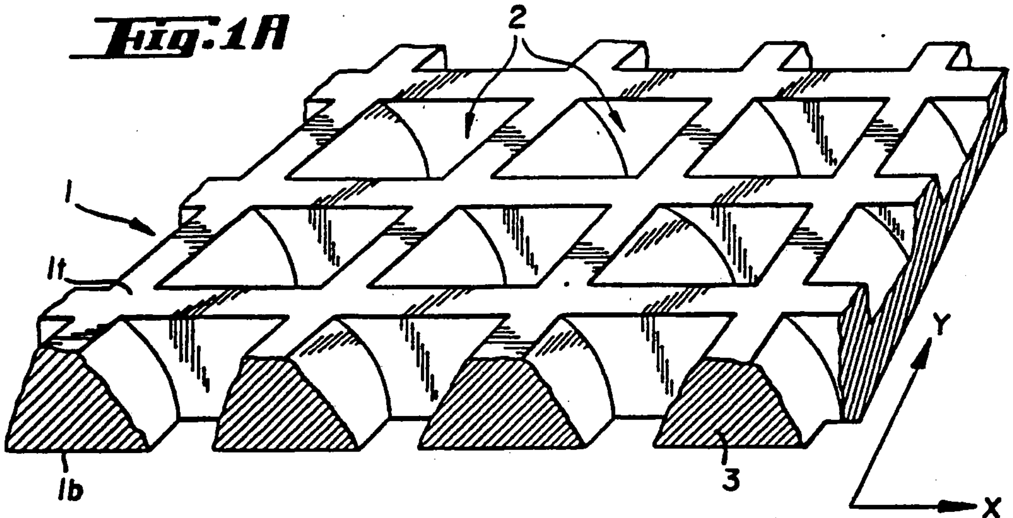
27. The carrier of Claim 24 wherein the base is metal and the coating is Si.

28. A method for forming a carrier for holding individual cells at identifiable locations comprising creating apertures in a base by subjecting the base to ion bombardment through masks of different thicknesses, the apertures being in an ordered array and being sized to hold individual cells.

29. A carrier for holding individual cells at identifiable locations comprising a base having substantially parallel top and bottom surfaces and having a plurality of apertures therethrough, said apertures (a) being arranged in an ordered array, (b) being sized to hold individual cells, and (c) having side walls which extend between said top and bottom surfaces of said base, said side walls including a first portion which converges inward towards the center of the aperture and a second portion which is substantially perpendicular to said top and bottom surfaces of said base.

30. The carrier of Claim 29 wherein the apertures are formed by ion bombardment.

Fig. 1A



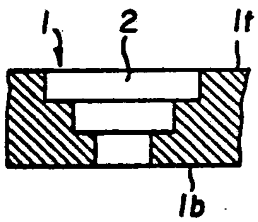


Fig. 1C

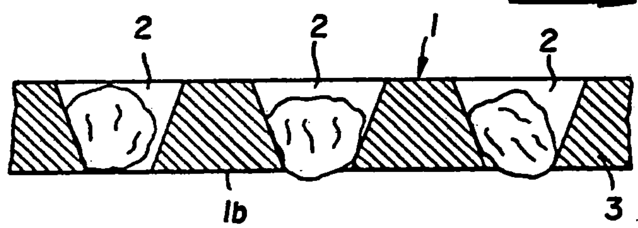


Fig. 1B

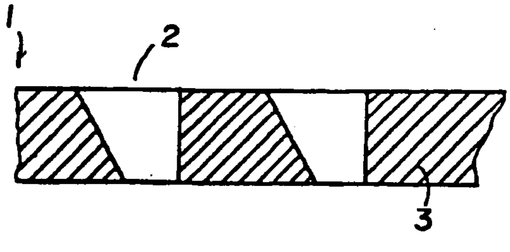


Fig. 1D

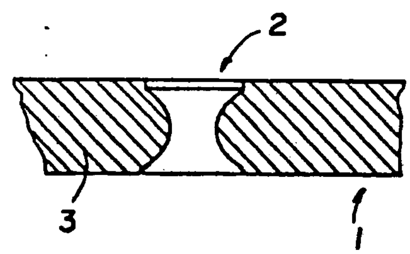


Fig. 1E

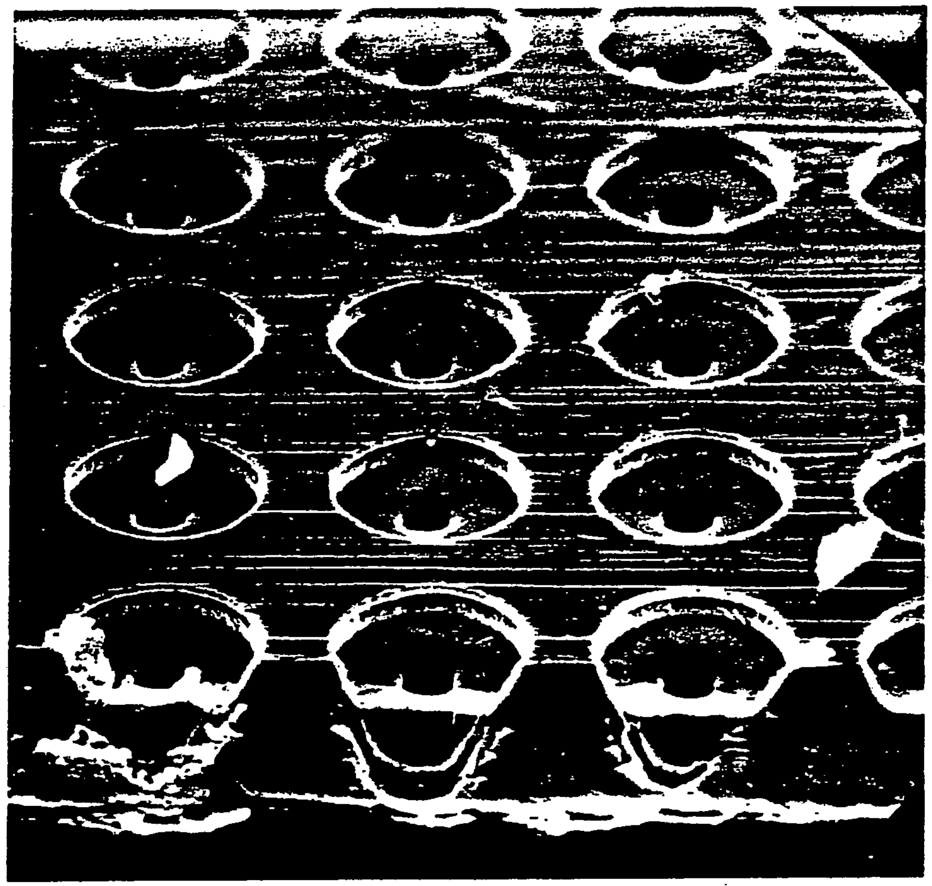


Fig. 2

SUBSTITUTE SHEET

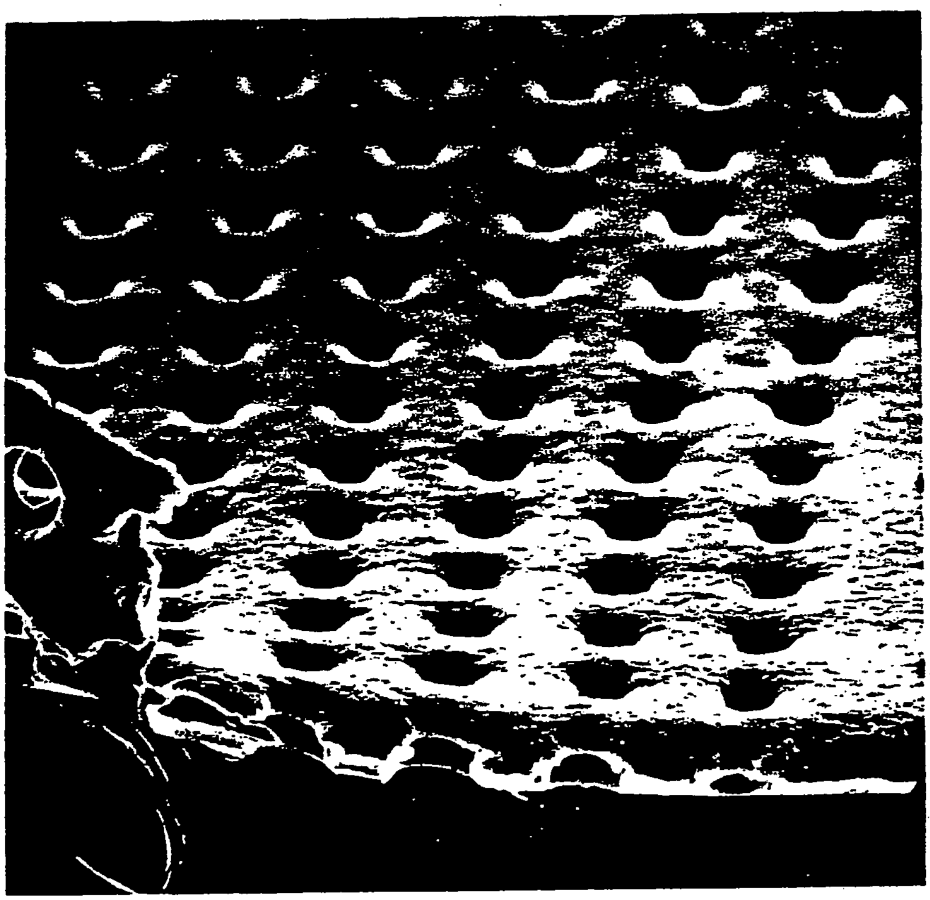


Fig. 3

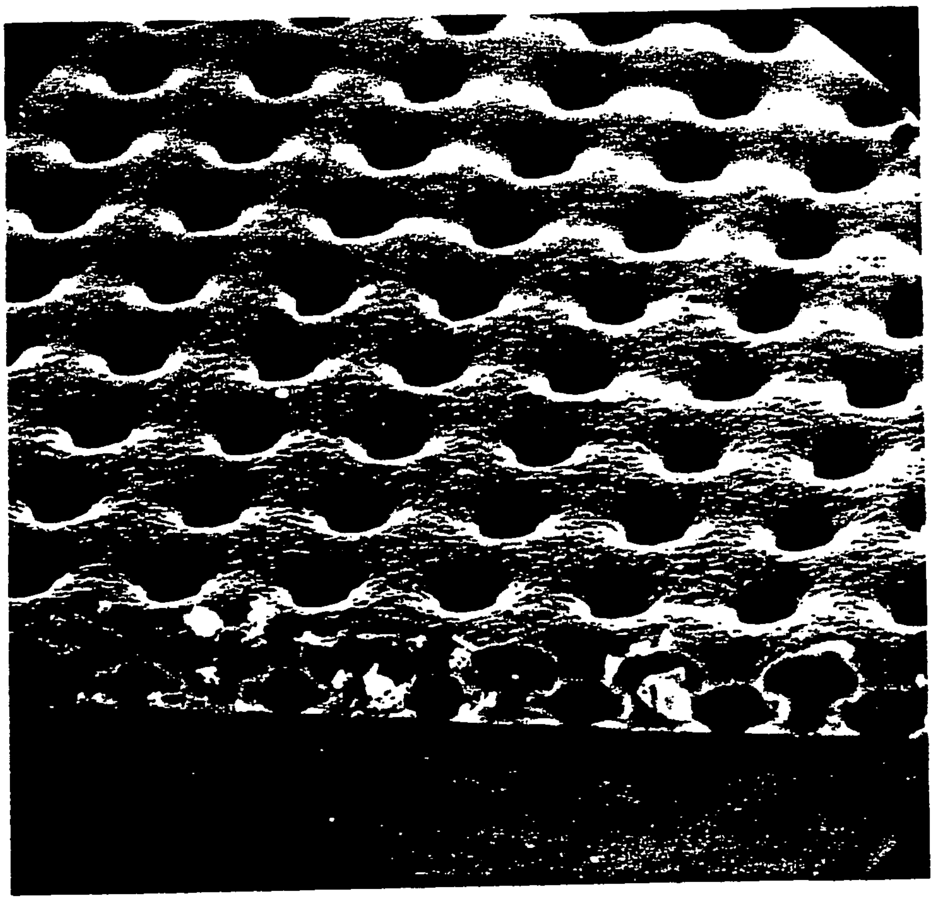


Fig. 4

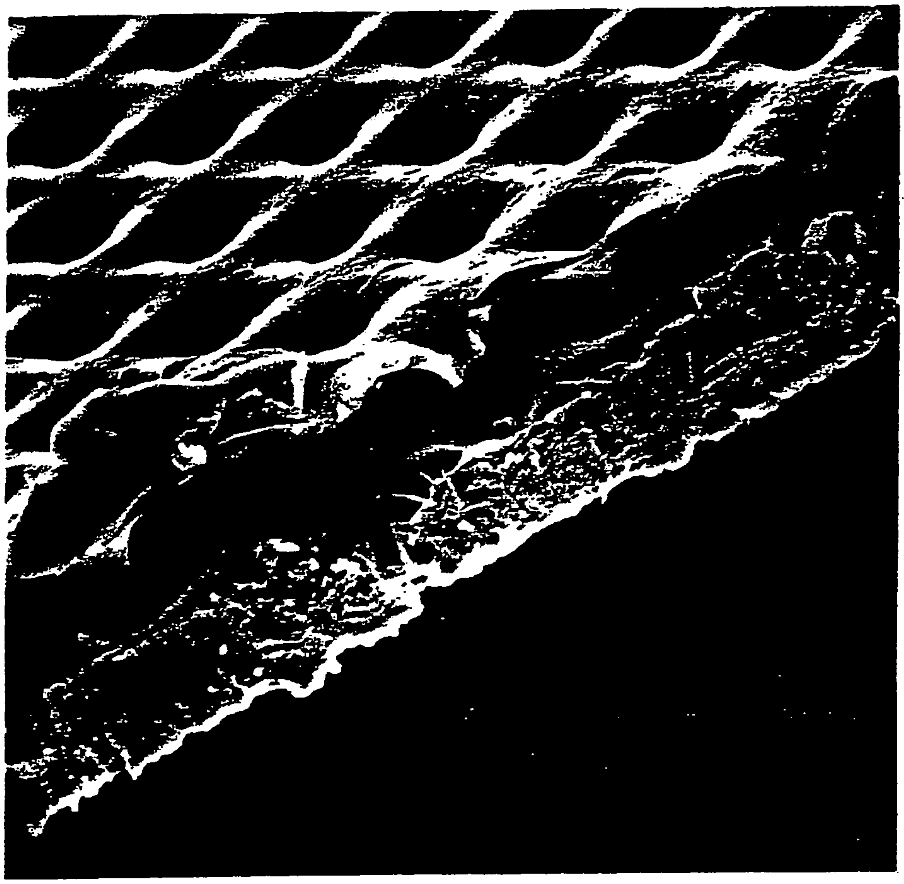


Fig. 5

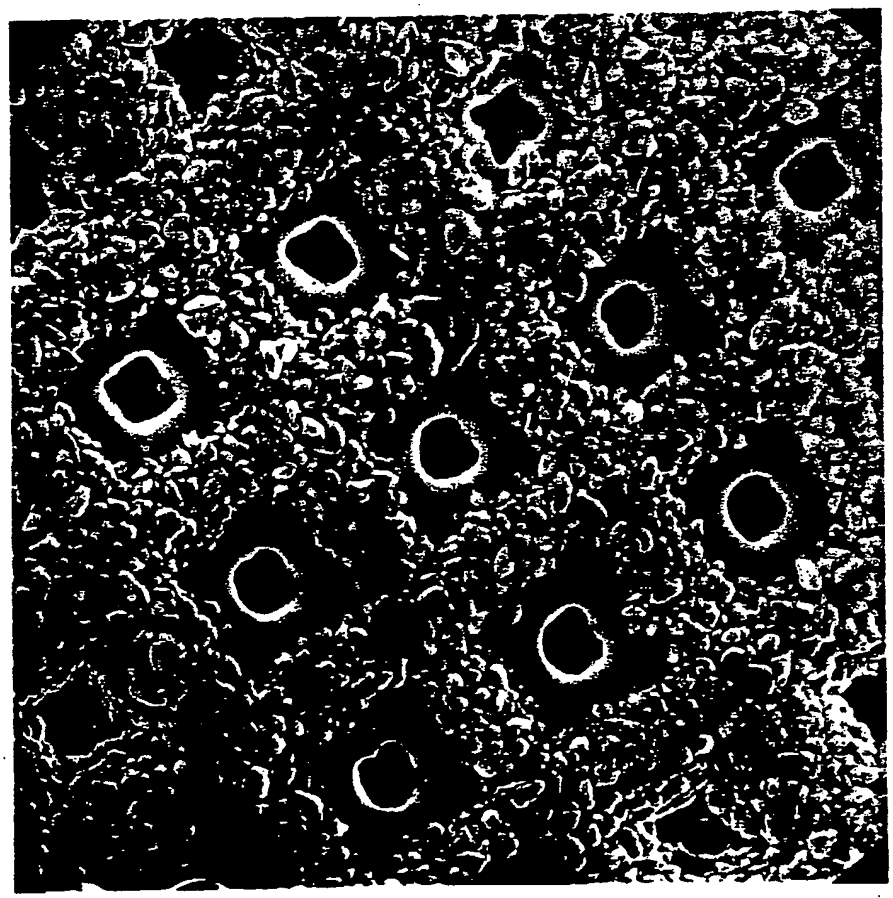


Fig. 6

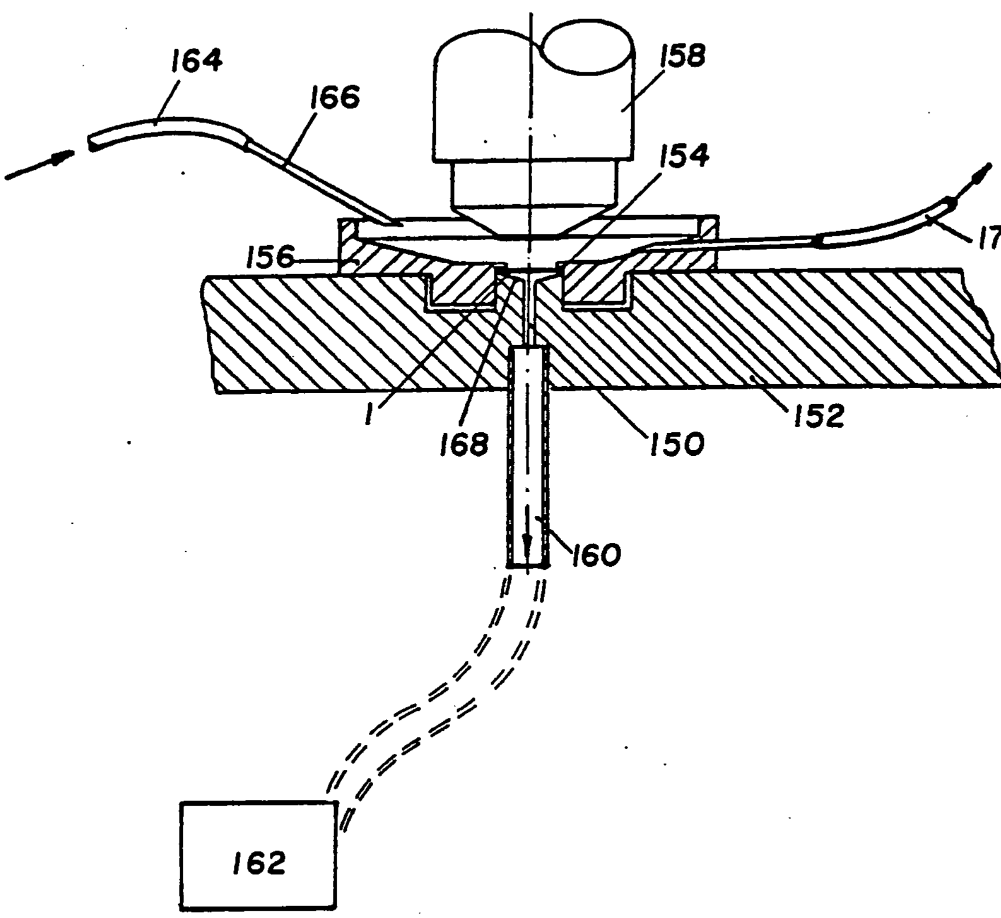


Fig. 7

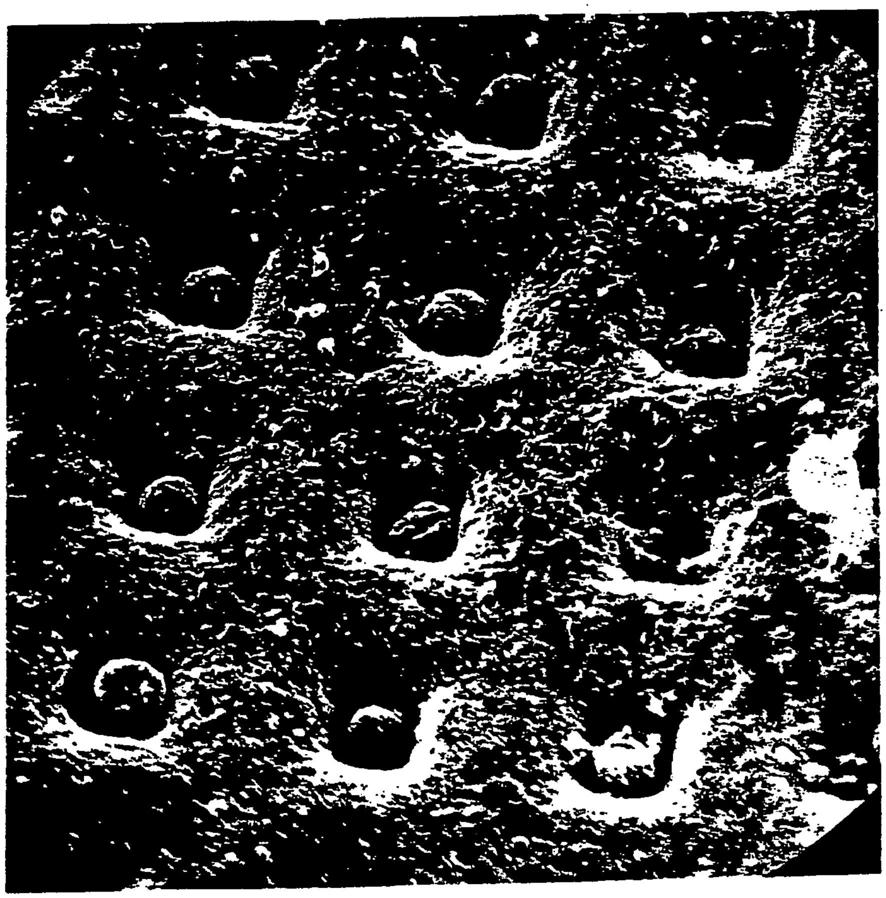


Fig. 8

SUBSTITUTE SHEET

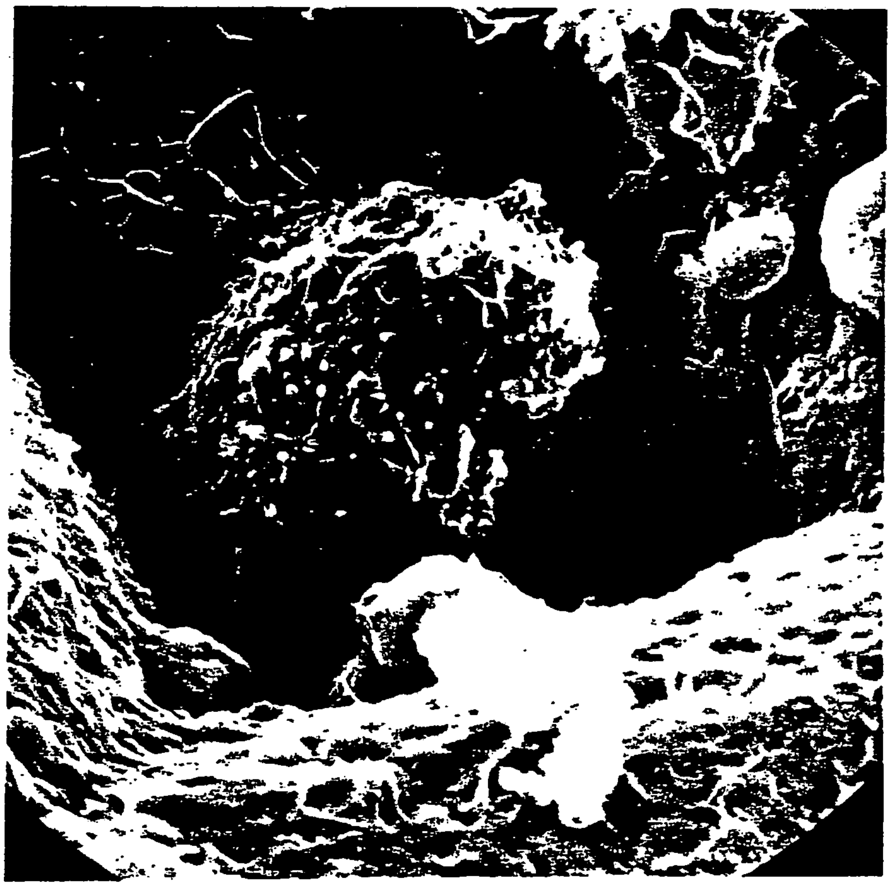


Fig. 9

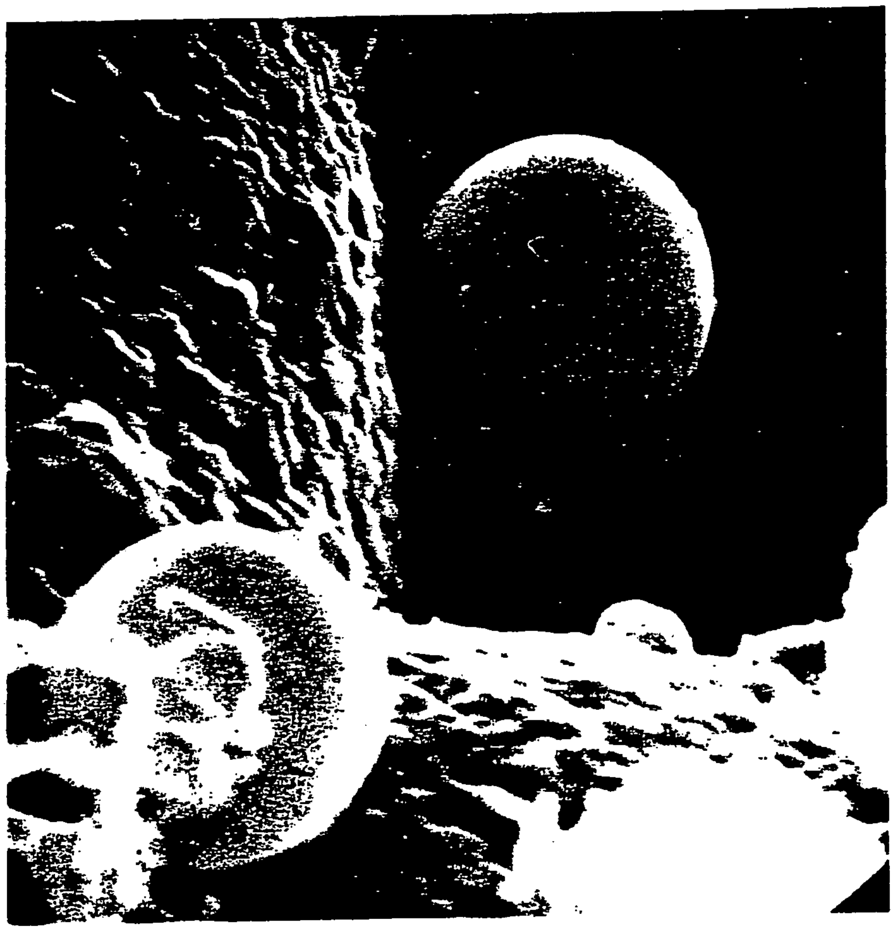


Fig. 10

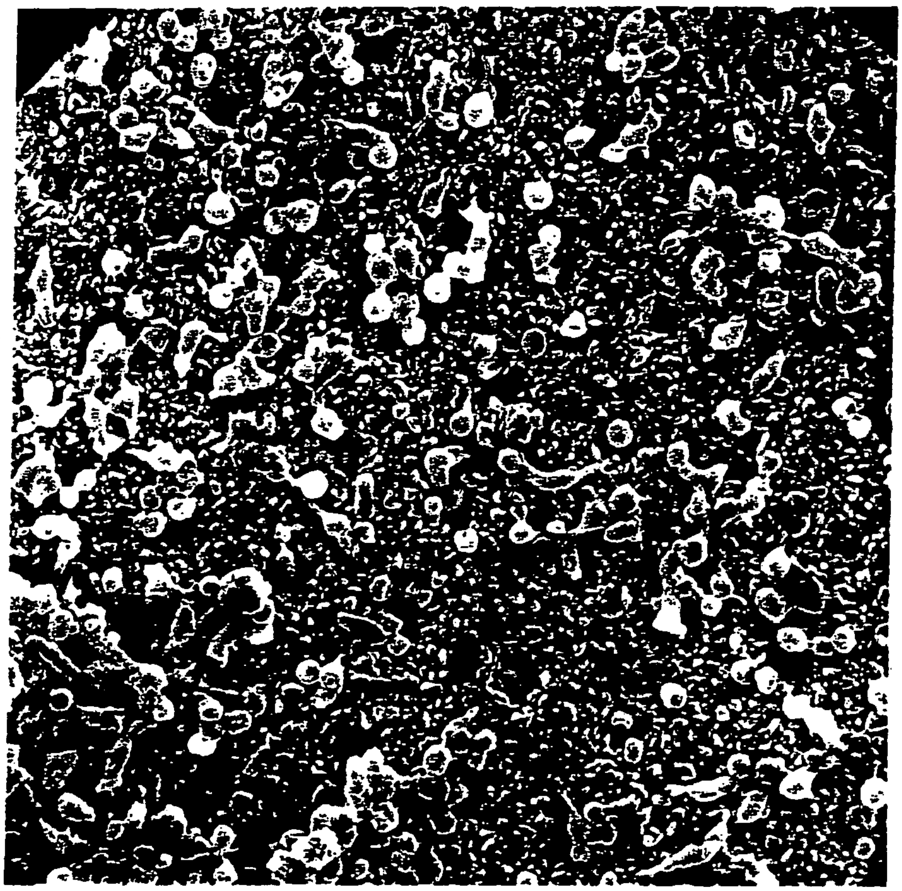


Fig. 11

SUBSTITUTE SHEET

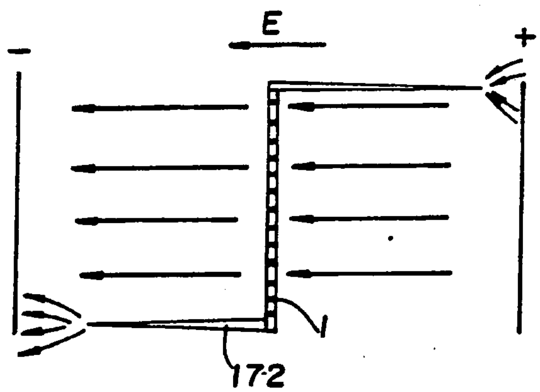


Fig. 12

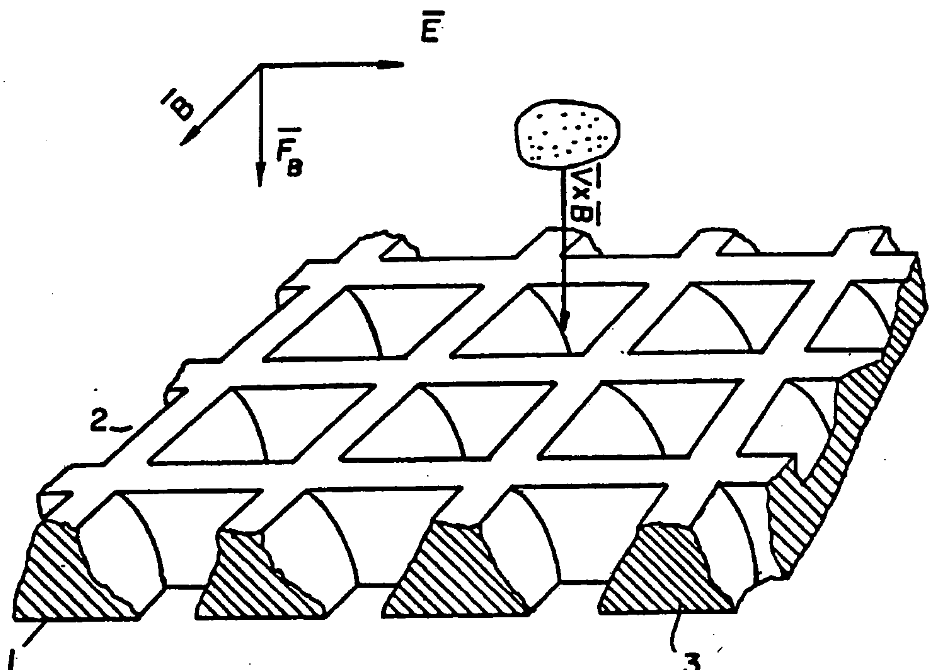


Fig. 13

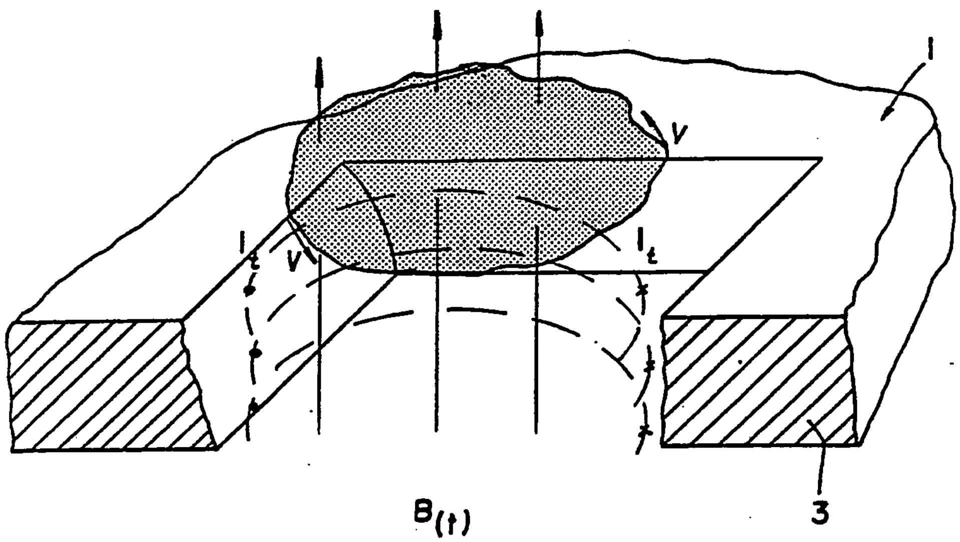


Fig. 14

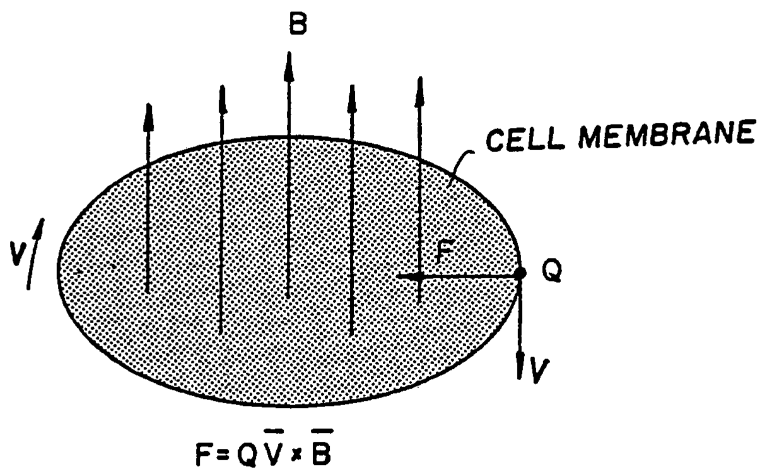


Fig. 15

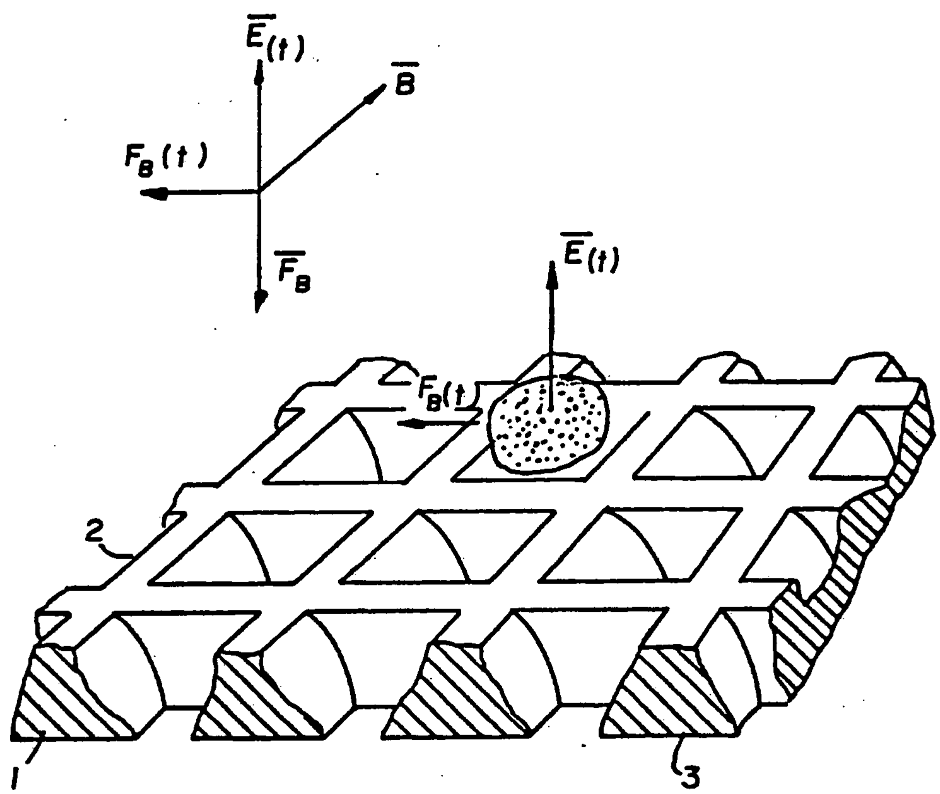


Fig. 16

INTERNATIONAL SEARCH REPORT

International Application No PCT/US84/01829

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

U.S. CL. 435/30,173,261,287,291,311,803;422/101;436/63,177,178 Cont. on next page

INT. CL.[3] C12Q 1/24;C12N 13/00,1/02;C12M 1/00,1/34,1/12;

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435/30,173,261,287,291,311,803; 422/101; 436/63,177 178 210/222,695; 209/38,243; 204/180R, 186,301,305

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Computer Search: Biological Abstracts and Chemical Abstracts Data Bases

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US,A, 4,055,799 (Coster et al) 25 October 1977	1-9,23-27,29, 30
A	N, Laboratory Equipment Digest, Volume 18, number 10, Issued October 1980, High-Voltage FFE Separates Cells Without Damage.	1-9, 13-16
Y	US,A, 3,874,851 (Wilkins et al) 1 April 1975	21, 22
A	US,A, 3,874,851 (Wilkins et al) 1 April 1975	1-9,23-27,29, 30
A	US,A, 4,162,850 (Warren) 31 July 1979	1-9,23-27,29 30
A	US,A 4,025,306 (Studer) 24 May 1977	1-9,23-27,29, 30
Y	US,A, 2,910,406 (Novak) 27 October 1959	23-27, 29, 30

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [3]
31 January 1985	07 FEB 1985
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	(Randall E. Deck)

Form PCT/ISA/210 (second sheet) (October 1981)

I. CLASSIFICATION OF SUBJECT MATTER:

US. 210/222, 695; 209/38, 243; 204/180R, 186, 301, 302, 305

INT. B01L 11/00; B01D 35/06, 57/02, 13/02; B03C 1/30, 1/02, 5/02

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	US,A, 2,910,406 (Novak) 27 October 1959	1-9
Y	US,A, 2,923,669 (Poitr s) 2 February 1960	21, 22
A	US,A, 1,915,568 (Gortner et al) 27 June 1933	1-9
A	US,A, 3,368,963 (Hall) 13 February 1968	1-9
A	US,A, 4,089,765 (Dudley) 16 May 1978	1-9

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ______, because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ______, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
A	US,A, 3,190,827 (Kok et al) 22 June 1965	1-9
Y	US,A, 3,207,684 (Dotts, Jr.) 21 September 1965	3,9,13,15
A	US,A, 4,374,644 (Armstrong) 22 February 1983	12
A	US,A, 3,929,583 (Sharpe et al) 30 December 1975	1,23-27,29, 30
A	US,A, 4,052,163 (Patzner) 4 October 1977	23-27,29,30
A,E	US,A, 4,441,972 (Pohl) 10 April 1984	1-9
A	US,A, 3,719,583 (Ustick) 6 March 1973	1-9
A	US,A, 4,326,934 (Pohl) 27 April 1982	1-9